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Influence of Commercial Processing Methods on Biochemical Diversity and Antioxidant Capacity of Pomegranate Juice

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Abstract: Although pomegranate is a polyphenol-rich fruit, processing conditions involved in the transformation of its matrix into juice can affect its polyphenol diversity and potential health benefits. This study investigated how 4 commercial pomegranate juices produced via different processes (i.e., concentrates, cold-pressed and presence of pulp) differed in polyphenol composition and antioxidant capacity. From our findings, ellagitannins (200.12 to 122.07 mg/L) and total anthocyanins (9.03 to 163.67 mg/L) were significantly ($p < 0.05$) prevalent in concentrate-developed juices, whereas total tannins, polymeric anthocyanins and total polyphenols were significantly retained in Cold-Pressed Pulp-containing juices. Additionally, cold-pressing conserved antioxidant capacity compared to juice production from concentrates. Overall POJU cold-pressed with pulp preserved antioxidant capacity by 40.38 and 97.65%, compared to POM Wonderful and Bolthouse Farms produced from concentrates, respectively. Overall, findings support cold pressing as an innovative technology for production of high-grade pomegranate juices. Thus, industrial application of cold-pressing can help produce more healthful beverages.

Key words: Pomegranate juice, ellagitannins, polyphenols, antioxidant activity, pomegranate.

1. Introduction

In recent times, consumers are becoming increasingly aware of the relationship between food consumption and positive health, thus, causing demand for natural foods that provide additional health benefits beside basic nutrition. Among such natural food groups are fruit juices, which are unfermented natural liquids harnessed after subjecting fruits to scientifically approved processing conditions outlined by Codex Alimentarius Commission. According to Statistica [1], the juice industry generated about US$ 131.08 billion in 2025. Annual sales of this revenue contributed by supermarkets and convenience stores are predicted to increase by 4.77% from 2025 to 2029. This data clearly demonstrates a high demand for fruit juices as a convenient dietary approach to meeting the intake of plant-based healthful metabolites (such as carotenoids, polyphenols, vitamins, organic acids and many others) required for proper growth, development, and wellness. Among such healthful juices is pomegranate fruit juice.

Pomegranate (*Punica granatum*) is a fruit belonging to the family *Punicaceae*, with a wide production geography in India, China, Iran and Turkey [2]. In recent times, pomegranate has received much popularity due to its rich composition of secondary metabolites (i.e., compounds that do not contribute to a plant's normal growth and development but serve as protective agents against environmental disruptions such as oxidative stress, pest infections and microbial invasions) that offer bioactive benefits required for maximum human health [3]. From previous research findings, presence of these secondary metabolites is responsible for pomegranate's appreciable health contributions (including antioxidant, anti-cancer, antidiabetic, anti-inflammatory, anti-tumor and many more) when

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present in diets.

Dominant secondary metabolites present in pomegranate juice include flavonoids (e.g., catechin, epicatechin, quercetin, luteolin and many others), phenolic acids (e.g., ferulic acid, gallic acid, ellagic acid, ferulic acid and others), anthocyanins (e.g., delphinidin 3.5-diglucoside, cyanidin 3.5-diglucoside, delphinidin 3-glucoside, and cyanidin 3-glucoside) and tannins (e.g., punicalagin and ellagitannin.) [4]. To support this claim is the extensive review authored by Fahmy et al. [2], where the authors reported on biochemical diversity of pomegranate juices and their associated health benefits.

However, it must be highlighted that apart from genetics, geographical origin, cultivation factors, and environmental conditions, the biochemical diversity and concentration of pomegranate's health promoting secondary metabolites also depend on processing conditions along the production chain. Many pomegranates juices available on the market are produced from reconstituted fruit concentrates, which are fruit juices with a significant amount of their water removed to form products higher in soluble solids. Concentrates of fruit juices enable the beverage industry to prolong shelf-life due to the reduction of water activity and microbial load. Production of juices from concentrates also helps the industry reduce transport and storage costs. Notwithstanding these benefits, the temperatures under which fruit juice concentrates are produced have been proven by research to have detrimental effects against retention of healthful native compounds and sensory supporting biomarkers [5]. Brunda et al. [6] reported on biochemical variations among pomegranate juices produced from concentrate and not-from-concentrate (i.e., freshly pressed). From their results, not-from-concentrate pomegranate juice exhibited higher antioxidant capacity, total polyphenol and anthocyanin contents by 10.07, 16.44 and 23.93 %, respectively, compared to their counterparts produced from concentrates. Another processing factor that contributes to biochemical variation and subsequent health properties in fruit juices is clarification (a production process where fruit juices are exposed to cell wall hydrolytic enzymes, centrifugation and filtration to remove turbidity-causing compounds for a clear juice yield). Supporting this literature is the work of Baklouti et al. [7] where the authors observed significant reductions of phenolic compounds (i.e., ellagic acid and punicalagin α/β) in clarified pomegranate juice, compared to their pulp-containing counterpart. The presence of pulp improves the nutritional and functional value of fruit juices because of their composition of fiber, glycosidic polyphenols, vitamins, minerals, organic acids and terpenes.

Although this information is crucial for guiding the industry toward the production of consumer-centric fruit juices, knowledge specific to pomegranate juice remains extremely limited. Thus, the objective of this study was to comparatively evaluate four commercial pomegranate juices differing in processing pathways, such as reconstitution from concentrate, cold pressing and the inclusion of pulp. We hypothesize that, commercial pomegranate juices (PJs) produced using non-thermal technologies such as cold pressing will retain a higher parentage of their biochemical profile compared to PJs produced using thermal technologies, which are capable of degrading heat-labile metabolites. To the best of our knowledge, this represents the first comparative investigation addressing these three production factors in pomegranate juices. Our findings will provide novel insights into how processing methods shape the polyphenol composition and antioxidant potential of commercial pomegranate juices, thereby informing both industry practices and consumer choices.

2. Materials and Methods

2.1 Pomegranate Juice (PJ)

Four 100% PJs (i.e., POM Wonderful, Red Crown, POJU, and Bolthouse Farms) were purchased from grocery shops in British Columbia, Canada. Red

Crown and POJU are produced by cold-pressing arils of pomegranate varieties indigenous to Azerbaijan. According to my knowledge, POM Wonderful and Bolthouse Farms are produced from reconstituted and pasteurized whole pomegranate juice concentrates obtained from California grown pomegranates. For pulp studies, the research focused on Red Crown and POJU because these were the investigated PJs with their pulp-containing options available.

2.2 Determination of Biochemical Diversity

2.2.1 Total Polyphenols

Total polyphenol content was determined as previously detailed by Miletic et al. [8], but with slight modifications. Polyphenols were initially extracted with acidified aqueous methanol, centrifuged and filtered with 0.45 µm syringe filter. Next, the obtained methanolic extracts were reacted with Folin-Ciocalteu reagent under alkaline conditions and incubated for 30 min at room temperature under darkness. Absorbance was measured at 765 nm after incubation. Quantitative evaluation of total polyphenols was calculated and expressed as mg/kg gallic acid equivalent using a standard curve prepared from pure gallic acid.

2.2.2 Total Anthocyanin

Total anthocyanin content was evaluated using HPLC-UV/Vis (High-Performance Liquid Chromatography/Ultraviolet-Visible Detection), as described by Gil et al. [9]. Briefly, anthocyanins were extracted with methanol and filtered through a 0.45 µm syringe filter before HPLC analysis. Afterwards, filtered samples were injected into a reverse-phase C18 column HPLC separation system. Detection was performed with UV-Vis at an absorbance of 540 nm. For quantitative results, total anthocyanin concentration was calculated using a calibrated curve prepared with monomeric anthocyanin standards, and expressed as mg/L.

2.2.3 Polymeric Anthocyanin

Polymeric anthocyanins were extracted and evaluated as described above for total anthocyanin. Concentrations of polymeric anthocyanins were evaluated and expressed in mg/L by using a standard calibration curve.

2.2.4 Catechins

Catechin from pomegranate juice was extracted using methanol and injected into an HPLC. Next the methanolic juice was centrifuged and filtered through a 0.45 µm syringe filter to obtain methanolic catechin extracts. Qualitative analysis was performed using HPLC built with a C18 column as the stationary phase. Detection of catechin was observed via UV-Vis absorption at 280 nm. Results were expressed in mg/L, using a standard curve developed from pure catechin [9].

2.2.5 Tannin

Tannin was extracted with methanol and evaluated via HPLC Detection observed with UV-Vis at an absorbance of 280 nm. Afterwards, quantification was performed using a calibration curve prepared from standard tannin, and expressed as mg/L [9].

2.2.6 Ellagitannins

Extracts of ellagitannins were prepared by subjecting pomegranate juice samples to acid hydrolysis to release ellagitannins as ellagic acid [10]. Briefly, aliquots of each pomegranate juice were mixed with methanolic concentrated hydrochloric acid and heated to release ellagitannins as ellagic acid. The obtained heated ellagic acid extracts were allowed to cool to room temperature and centrifuged afterwards. Next, the obtained supernatant was passed through a 0.45 µm PVDF (Polyvinylidene Fluoride) syringe filter before being subjected to separation via RP-HPLC (Reversed Phase-High-Performance Liquid Chromatography). Detection of ellagic acid was performed with a UV-Vis diode-array detector at 360 nm. Quantification was performed by using a standard calibration curve prepared from pure ellagic acid, and expressed as mg/L.

2.2.7 Evaluation of Antioxidant Activity

Antioxidant capacity of commercial pomegranate juice was assessed using DPPH (2, 2-Diphenyl-1-picrylhydrazyl) assay as described by AOAC (Association of Official Analytical Chemists) [11]. Briefly, aliquots of juice polyphenol extract were reacted with a DPPH solution and kept under darkness for 30 minutes at room temperature. After DPPH reaction time, absorbance of the reactive solution was taken at 517 nm with a spectrophotometer. Trolox was used to generate a standard curve, and antioxidant capacity was expressed as micromoles Trolox equivalents per 100 g of sample.

2.3 Statistical Analysis

All analytical studies were conducted in triplicates, with results presented as mean ± standard deviation. Data entry was performed in Microsoft Excel (Microsoft Corporation, Redmond, WA, USA; Microsoft 365 version). To evaluate significant differences among mean values, a one-way ANOVA (Analysis of Variance) with Bonferroni post-hoc tests was performed using Python (Python Software Foundation, Version 3.11) with the SciPy and statsmodels libraries. Principal Component Analysis was performed using Python (Version 3.11) with the scikit-learn library.

3. Results and Discussion

3.1 Biochemical Composition of PJs from Cold-pressing and Reconstituted Concentrates

The health benefits of PJ are directly proportional to its composition of secondary metabolites. Thus, in this study, mean values of healthful secondary metabolites profiled in commercial PJ are presented in Table 1. Apart from catechin, significant differences ($p < 0.05$) were found among biochemical composition of commercial PJs. The highest level of ellagitannin was reported with POM Wonderful at 200.12 mg/L followed by POJU Cold-Pressed > Red Crown Cold-Pressed > Red Crown Cold-Pressed/Pulp > POJU Cold-Pressed/Pulp > Bolthouse Farms. This observation with POM Wonderful was significantly higher ($p < 0.05$) by 22.55, 23.55, 26.04, 38.04 and 39.00 % compared to POJU Cold-Pressed, Red Crown Cold-Pressed, Red Crown Cold-Pressed/Pulp, POJU Cold-Pressed/Pulp and Bolthouse Farms. According to our knowledge, POM Wonderful is produced from concentrates obtained from whole pomegranate fruits including its arils, rind and membranes. Previous studies have reported pomegranate rind as the structural tissue with the highest deposition of ellagitannins.

Thus, accounting for POM Wonderful’s higher ellagitannin content than with Red Crown and POJU which is obtained from only cold-pressed pomegranate arils with their rind portion mostly removed. The range of ellagitannin observed in this study is similar and even higher than in PJs produced from different Iranian cultivars, where ellagitannin concentration ranged between 7 to160 mg/L [12].

Unlike ellagitannins, results for polymeric anthocyanins and total tannins exhibited a distinct trend, with concentrations ranging from 4.00-9.00 mg/L and 105.07-220.00 mg/L, respectively. Among the investigated PJs, Red Crown Cold-Pressed/Pulp recorded the highest mean values for both polymeric anthocyanins and total tannins. In contrast, Bolthouse Farms and POJU Cold-Pressed showed the lowest mean levels, which were 55.56 and 52.24 % lower, respectively, than those of Red Crown Cold-Pressed/Pulp. By structural comparison, literature reports tannins to be highly deposited in pomegranate rind than other structural components of the fruit.

However, this narrative was not reflected in our data where the highest mean value for total tannins was recorded with Red Crown Cold-Pressed/Pulp (produced from arils only) than with POM Wonderful (produced from whole fruit concentrate). This may be due to the presence of hydrolyzable tannins tightly bound to insoluble structural cell wall components forming the rind of whole pomegranate fruits used, thus, making POM Wonderful tannins insoluble for detection compared to Red Crown Cold-Pressed/Pulp.

Table 1 Impact of processing methods on biochemical composition of commercial PJs.

PJ brand	Ellagitannins mg/L	Catechin mg/L	Total tannins mg/L	Polymeric anthocyanins mg/L	Total anthocyanins mg/L	Total polyphenols mg/kg
POM Wonderful	200.12 ± 0.13[a]	0.99 ± 0.00[a]	132.80 ± 0.20[c]	5.00 ± 0.10[c]	163.67 ± 0.61[a]	1,900.00 ± 0.26[e]
Red Crown Cold-Pressed	153.00 ± 0.10[c]	0.99 ± 0.00[a]	194.03 ± 0.12[b]	7.00 ± 0.10[b]	7.10 ± 0.10[f]	2,456.00 ± 0.20[d]
Red Crown Cold-Pressed/Pulp	148.00 ± 0.10[d]	0.99 ± 0.00[a]	220.00 ± 0.20[a]	9.00 ± 0.10[a]	9.03 ± 0.61[e]	2,577.00 ± 0.20[c]
POJU Cold-Pressed	155.00 ± 0.10[b]	0.99 ± 0.00[a]	105.07 ± 0.15[f]	5.00 ± 0.10[c]	68.03 ± 0.15[c]	2,699.92 ± 0.19[b]
POJU Cold-Pressed/Pulp	124.00 ± 0.10[e]	0.99 ± 0.00[a]	123.93 ± 0.15[e]	5.00 ± 0.11[c]	71.03 ± 0.10[d]	3,000.00 ± 0.19[a]
Bolthouse Farms	122.07 ± 0.21[f]	0.99 ± 0.00[a]	128.07 ± 0.15[d]	4.00 ± 0.17[d]	125.07 ± 0.15[b]	2,700.07 ± 0.32[b]

Data are presented as mean ± SD of three independent experiments. Different superscript letters within a column indicate significant differences ($p < 0.05$). PJ: Pomegranate Juice.

Furthermore, the release of polyphenol oxidase (i.e., the enzyme responsible for oxidizing polyphenols into o-quinones, which subsequently interact with anthocyanins to degrade the characteristic red hue in foods) from fruit rind during production, along with heat exposure during concentrate formation may be responsible for reduced tannins observed with POM Wonderful than Red Crown Cold-Pressed/Pulp.

Presence of total anthocyanin showed significant ($p < 0.05$) concentrations in the order POM Wonderful > Bolthouse Farms > POJU Cold-Pressed/Pulp > POJU Cold-Pressed > Red Crown Cold-Pressed/Pulp > Red Crown Cold-Pressed. From previous studies [13] anthocyanins are mainly deposited in the fruit's arils; thus, it's expected for cold-pressed pomegranate juice to retain higher anthocyanins. Notwithstanding, the trend observed with this study is interesting with reconstituted juice from whole-fruit pomegranate concentrate (POM Wonderful) reporting higher levels of anthocyanins than their cold-pressed counterparts. This may be due to the concentration effect of water removal during concentrate production, improved extraction of anthocyanins from rind tissues during thermal processing of concentrate, and the possible protective interactions with rind ellagitannins. Another explanation for this trend is the analytical method used for detection, which may have detected polymerized anthocyanin-tannin complexes reported to be more prevalent in concentrate than pressed juice.

While it's important to highlight that all investigated commercial PJs displayed the same concentration of catechin with no significant differences ($p > 0.05$), total polyphenols reported mean concentration values with significant differences ($p > 0.05$) among PJs. Overall POJU Cold-Pressed/Pulp presented the prevalent level of total polyphenols (3,000.00 mg GAE/kg), among studied commercial PJs. Compared with commercial PJs produced from concentrates, total polyphenols reported with POJU Cold-Pressed/Pulp was significantly ($p < 0.05$) higher than observed with Bolthouse Farms and POM Wonderful by about 1.111 and 1.579 times, respectively. This observation with pomegranate juices formulated from concentrates may be attributed to the thermal conditions associated with fruit-juice concentrate production. According to Silva and Anantheswaran [14], cold-pressing presents environmental conditions capable of arresting activities of polyphenol oxidizing factors such as polyphenol oxidase, peroxidase, heat, oxygen, light and microbial activities, thus, collectively enhancing the stability and retention of polyphenolic compounds. Additionally, differences in geographical origin and genetic pool of varietal differences may account for variations in reported total polyphenols.

It's important to highlight that, total polyphenol reported for all investigated commercial PJs was higher compared to previous observations documented by Gil et al. [9] and Mousavinejad et al. [12], where the authors reported PJs with total polyphenol content of 2,000 and 1,500 to 2,500 mg GAE/L, respectively. However, a similar range of 2,602 mg GAE/L for total polyphenol was observed with seven Turkey produced commercial PJs [15].

Overall, differences in biochemical compounds reported among the commercial PJs investigated in our study could be due to the differences in pomegranate cultivars used for juice extraction, differences in cultivation conditions, and differences in fruit maturity harvest. To support this explanation is the work of Labbé et al. [16]. Labbé and colleagues [16], evaluated anthocyanin profile and total phenolic content of juices from three pomegranate varieties, harvested at different maturity stages (i.e., unripe, medium ripe and full ripe). From their results, they concluded that although maturity stage did not significantly influence accumulation of total phenolic content, cultivar significantly played a role in the accumulation of polyphenols due to its genetic influence. Furthermore, processing conditions including pasteurization before bottling can also oxidize heat sensitive compounds such as anthocyanins, ellagitannins, catechin and other polyphenols, thus, contributing to reductions and variations among different commercially produced PJs.

3.2 Impact of Pulp Inclusion on Biomarkers of Cold-Pressed Pomegranate Juices (PJ)

For optimum health, it is recommended to consume whole fruit juice because the pulp fraction provides fiber, glycosidic-bound polyphenols, some vitamins and minerals. To study the variability of pulp's biochemical composition, pulp-containing PJs from Red Crown and POJU were further investigated as displayed on Table 2.

Except for ellagitannins and catechins, all other investigated secondary metabolites showed higher deposition in pulp-containing juices than their clarified alternatives. Ellagitannins are plant-based polyphenols broken down into ellagic acid by gut microbiome for antioxidant, anticancer, anti-cardiovascular and other health promoting purposes [17]. The distribution of ellagitannins among the commercial pomegranate juices was particularly noteworthy, following the order POJU Cold-Pressed > Red Crown Cold-Pressed > Red Crown Cold-Pressed/Pulp > POJU Cold-Pressed/Pulp. This trend showed that cold-pressed pomegranate juice with-no-pulp contained higher concentrations of ellagitannins than their pulp-containing counterparts. According to literature, ellagitannins are dominant in pomegranate structural membranes than their arils, meaning, it will theoretically be expected for pulp-containing PJ to have higher levels of ellagitannins, compared to their no-pulp alternative. Nonetheless, this study observed a different trend, where no-pulp cold-pressed Red Crown and POJU PJs were significantly ($p < 0.05$) higher in ellagitannins than their pulp-containing cold-pressed counterparts. This interesting trend may be attributed to differences in matrix effects, as no-pulp juices lack the presence of insoluble fiber-rich pulp entrapped with ellagitannins, thereby improving their extractability and detection in no-pulp juices [18].

For total tannins, Cold-Pressed Pulp-containing PJs presented significant levels, compared to their cold-pressed no-pulp forms. Cold-Pressed Red Crown Pulp-containing presented a 52.24 % significant ($p < 0.05$) concentration of total tannin, compared to Cold-Pressed POJU Pulp-containing PJ. Polymeric anthocyanins are stable anthocyanins formed when monomeric anthocyanins react with other phenolic compounds like tannins to form stable complex compounds. Similar to total tannins, polymeric anthocyanins were prevalent in Cold-Pressed Pulp-containing Red Crown than with Cold-Pressed Pulp-containing POJU. Polymeric anthocyanin in Cold-Pressed Red Crown pulp-containing juice was 1.8 times higher than with Cold-Pressed POJU Pulp-containing juice. Thus, Cold-Pressed Red Crown Pulp-containing PJ may be postulated to be more stable and resistant to color oxidation along post-process storage.

Table 2 Influence of pulp inclusion on biochemical composition of commercial cold-pressed PJs.

PJ brand	Ellagitannins mg/L	Catechin mg/L	Total tannins mg/L	Polymeric anthocyanins mg/L	Total anthocyanins mg/L	Total polyphenols mg/kg
Red Crown Cold-Pressed/Pulp	148.00 ± 0.10^{c}	0.99 ± 0.00^{a}	220.00 ± 0.20^{a}	9.00 ± 0.10^{a}	9.03 ± 0.61^{c}	2,577.00 ± 0.20^{c}
Red Crown Cold-Pressed	153.00 ± 0.10^{b}	0.99 ± 0.00^{a}	194.03 ± 0.12^{b}	7.00 ± 0.10^{b}	7.10 ± 0.10^{d}	2,456.00 ± 0.20^{d}
POJU Cold-Pressed/Pulp	124.00 ± 0.10^{d}	0.99 ± 0.00^{a}	123.93 ± 0.15^{c}	5.00 ± 0.11^{c}	71.03 ± 0.10^{a}	3,000.00 ± 0.19^{a}
POJU Cold-Pressed	155.00 ± 0.10^{a}	0.99 ± 0.00^{a}	105.07 ± 0.15^{d}	5.00 ± 0.10^{c}	68.03 ± 0.15^{b}	2,699.92 ± 0.19^{b}

Data are presented as mean ± SD of three independent experiments. Different superscript letters within a column indicate significant differences ($p < 0.05$). PJ: Pomegranate Juice.

With reference to total anthocyanins, Cold-Pressed POJU Pulp-containing juice reported 87.29 % higher levels compared to Cold-Pressed Red Crown Pulp-containing juice. Although monomeric anthocyanins are less stable than their polymeric forms, they're reported by literature to be more bioavailable for bodily use due to their smaller molecular size [19]. Considering *in-vitro* results of the current study, Cold-Pressed POJU Pulp-containing juice may be postulated to be more effective when considering anthocyanin-related health functionalities.

Lastly, results for total polyphenols were in synchrony with total anthocyanins, where Cold-Pressed Pulp-containing POJU recorded the highest mean value (3,000 mg GAE/kg), with this observation being significantly higher than reported for Cold-Pressed Red Crown Pulp-containing juice by 14.1%. It's also important to emphasize that, Cold-Pressed Pulp-containing Red Crown and POJU pomegranate juices were 4.70% and 10.03% higher in total polyphenols compared to their respective no-pulp containing PJs. Overall, Cold-Pressed PJs with pulp exhibited higher polyphenol levels than their non-pulp counterparts. This observation may be attributed to the retention of fiber-bound phenolic compounds, suspended solids and colloidal materials in the cloudy matrix of pulp-containing PJs. As reported by Carlsen et al. [20], clarification (i.e., the process of removing suspended solids, pulp and colloidal particles from juice to obtain a clear, transparent liquid) leads to the removal of fiber-bound phenolics, thereby, lowering total polyphenol content of PJs without pulp inclusion. A further advantage with pulp is its protective cushion role that helps stabilize polyphenols against enzymatic and oxidative degradation [21].

Additionally, the range of total polyphenols recorded with this study is comparable to observations reported by Nuncio-Jáuregui et al. [22] and Esposto et al. [23] on commercial PJs, where the authors reported PJs produced from rind, membranes and arils with total polyphenol ranges of 2,285-2,457 and 1,350-3,260 mg GAE/kg, respectively. Notwithstanding, results reported for our study showed lower ranges compared to some previous works. For instance, Milošević et al. [24] reported on pulp-containing PJs with total polyphenol range of 383.4 to 744.2 mg GAE/g. Variations in literature confirm our previous discussion that concentration of secondary metabolites in pomegranate is influenced by different pre- and post-harvest processing conditions crucial for their maximum retention along the production chain to the final consumer's table.

3.3 Antioxidant Capacity

Antioxidant capacity of a molecule is the molecule's ability to protect cells against oxidative stress caused by the presence of unstable free radicals. Antioxidant capacity involves different mechanism; thus, in this study the antioxidant capacity of commercial PJs was evaluated by testing its ability to donate hydrogen atom to an unstable DPPH free radical. From Tables 3 and 4, all cold-pressed PJs showed higher antioxidant activity than PJs made from concentrates.

Table 3 Impact of processing method on antioxidant capacity (μmol TE/100 g) of commercial PJs.

Bolthouse Farms	POM Wonderful	Red Crown CP	Red Crown CP/P	POJU CP	POJU CP/P
122.07 ± 0.21^{f}	3,100.00 ± 0.21^{e}	4,400.00 ± 0.50^{d}	4,600.00 ± 0.50^{c}	4,900.03 ± 0.50^{b}	5,200.00 ± 0.50^{a}

Data are presented as mean ± SD of three independent experiments. Different superscript letters within a row indicate significant differences ($p < 0.05$). CP: Cold-Pressed, CP/P: Cold-Pressed with Pulp.

Table 4 Effect of pulp inclusion on antioxidant capacity of commercial cold-pressed PJs.

Red Crown CP/P	Red Crown CP	POJU CP/P	POJU CP
4,600.00 ± 0.50^{c}	4,400.00 ± 0.50^{d}	5,200.00 ± 0.50^{a}	4,900.03 ± 0.50^{b}

Data are presented as mean ± SD of three independent experiments. Different superscript letters within a row indicate significant differences ($p < 0.05$). CP/P: Cold-pressed with pulp, CP: Cold-pressed with no pulp.

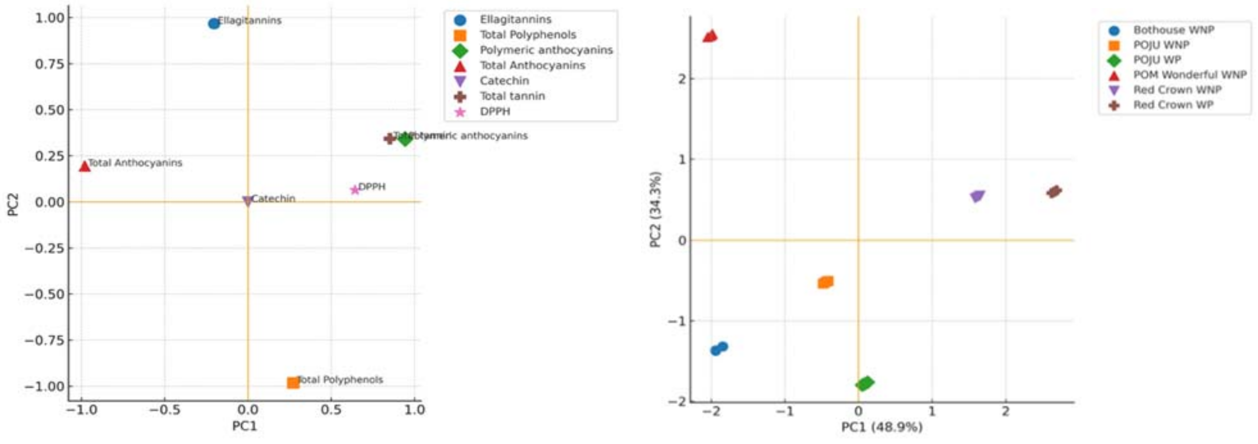
Cold-Pressed POJU Pulp-containing pomegranate juice recorded the highest antioxidant activity of 5,200.00 μmol TE/100 g followed by Cold-Pressed POJU with-no-pulp juice at 4,900.03 μmol TE/100 g. The antioxidant capacity of pulp-containing Cold-Pressed POJU was 40.38% and 97.65% significantly ($p < 0.05$) higher than POM Wonderful and Bolthouse PJs formed from concentrates, respectively. This trend is in synchrony with our results for total polyphenols, where cold-pressed juices were higher in total polyphenol content than their concentrate formulated PJ alternatives. Another interesting observation was Bolthouse Farms recording the lowest antioxidant capacity, although it showed significant levels of total polyphenols than recorded for cold-pressed Red Crown with and without pulp. This trend is because the antioxidant capacity of a food is not always simultaneous with its polyphenol content but rather dependent on the individual types of polyphenols present in the food matrix, concentration of each type of polyphenol compound, the number of functional groups present in the polyphenol compound for antioxidant functionalities, interference of other interactive compounds in the reaction medium or food matrix, and bio-accessibility and bioavailability of the types of polyphenols involved in the antioxidant chain [25]. Additionally, low temperature, oxygen control and arrest of phenolic degradative enzymes under cold-pressing enable protection and retention of polyphenols for antioxidant performance, whereas the high heat treatment involved with concentrate production leads to thermal oxidation, degradation of polyphenols and reduced antioxidant performance.

3.4 Variability Between Commercial Pomegranate Juice and Their Biochemical Composition via Principal Component Analysis

PCA (Principal Component Analysis) was performed to evaluate visual variability among commercial PJs based on their biochemical diversity. Score and loading plots (Fig. 1) were used to determine the relationship between commercial PJs and how their production from cold-pressing or concentrates, as well as the presence of pulp influences their biochemical composition and antioxidant activity.

From Fig. 1a, PC1 reflected variation in total polyphenols, ellagitannins, and total tannins, a showing strong positive loading on this axis. Cold-Pressed Pulp-containing POJU and Red Crown were clustered at the positive end of PC1 (Fig. 1b). The separate clustering of Cold-Pressed Pulp-containing PJs is due to their higher concentration of total polyphenols and total tannins. According to literature, presence of pulp contributes to the food matrices polyphenol advantage due to the release of polyphenols originally bound to structural molecules such as carbohydrates and proteins [26].

From the scoring plot, there's also the clustering of total tannins and polymeric anthocyanins on the positive axis of PC1, which showed a strong correlation with



(a) (b)

Fig. 1 PCA score plot and loading plots of principal components for biochemical diversity (a) and commercial pomegranate juices (b). WP: With Pulp, WNP: With No Pulp.

Cold-Pressed Pulp-containing Red Crown at the positive side of PC2. This confirms our results presented in Table 2, where statistical analysis identified Cold-Pressed Pulp-containing Red Crown as dominant in both total tannins and polymeric anthocyanins. Another visual observation from the PCA was the close correlation of POM Wonderful juice from concentrate with the positive axis of PC2, which correlated with ellagitannins on the positive axis of PC1. This confirms our discussion at Section 3.1. which presented prevalent levels of ellagitannins in POM Wonderful PJ produced from concentrates developed from the rind, membranes and arils of the pomegranate fruit than in cold-pressed PJs developed from the fruit's arils only, thus, supporting previous works that ellagitannins are structurally prevalent in the fruit's rind than arils.

Furthermore, Cold-Pressed Pulp-containing Red Crown juice (PC2) showed a strong association with polymeric anthocyanins and total tannins (PC1) on the positive axis of PC1, confirming our initial postulation of its capacity to retain color stability while exerting significant antioxidant activity than with POM Wonderful and Bolthouse Farms produced from concentrates. Overall, separation from PCA displays how processing conditions (i.e., concentrate, cold-processed, and pulp inclusion), jointly shape the biochemical composition, antioxidant capacity and pigment stability of commercial PJs.

4. Conclusion and Future Perspective

The key finding of this study is that, the concentration of biochemical compounds and their related antioxidant activity is strongly dependent on the processing methods applied along the production chain. From this study, Cold-Processed PJs showed significant antioxidant capacity due to the protection of their polyphenols from oxidation compared to PJs produced from concentrates. It is also worth mentioning that our results demonstrate that antioxidant capacity of commercial PJs is not solely dependent on polyphenol concentration, but also by the specific types of polyphenolic compounds present in the PJ matrix. Additionally, Cold-Pressed Pulp-containing PJs showed prevalent composition of polyphenols with significant reflection in antioxidant capacity than observed with cold-pressed no-pulp containing PJs and PJs produced from concentrates.

Given that most consumers rely on commercially available PJs rather than home-prepared options, these findings highlight the importance for industry to prioritize the production of cold-pressed PJs with inclusion of pulp membranes over the production of PJs from concentrates. Thereby, for future efforts aimed at retaining antioxidant and other health-promoting properties of PJ, processing innovations such as pre-treatment (i.e., rind, membranes and arils, or desired fruit portion depending on the PJs market target) with ultrasound, high-pressure processing and pulsed electric field before cold-pressing will enhance loosening of bound structural membranes for improved juice extraction, polyphenol release, oxidative enzyme deactivation, microbial inactivation, polyphenol stability and health benefits. However, these advanced technologies are expensive, and thus, will require in-depth cost-effective analysis before industrial application. Furthermore, to better understand how PJ bioactive compounds affect consumer health, in-vivo studies of their effect on different metabolic pathways should be exploited to guide the industry's product development applications.

Disclosure Statement

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